**Remarks/Arguments**

Claim 1 is currently amended to recite that a semi-solid culture medium is supported by a solid phase and comprises a pre-incubated mixture of at least one bacterial strain and at least one phage type, and a hydrocolloid at a concentration below 0.3%. Claim 1 is further currently amended to recite that the crude bacteriophage extract is obtained by sequential serial extractions, the titer of the crude bacteriophage extract is at least $10^{11}$ pfu/ml, and the bacteriophage yield is at least in the order of magnitude of $10^{15}$ to $10^{16}$ total pfu.

Claims 4, 9 and 10 are now canceled, and claims 11, 12 and 14 are amended to change their dependency from canceled claim 10 to claim 1.

New claim 53 recites that the sequential serial extraction comprises the steps of:

(a) collecting the semi-solid culture medium;

(b) adding fresh medium to the semi-solid culture medium to obtain a slurry;

(c) mixing the slurry intensively;

(d) centrifuging the slurry to obtain a supernatant comprising a crude bacteriophage extract;

(e) collecting the obtained crude bacteriophage extract; and

(f) repeating the aforementioned steps until the phage titer in the crude bacteriophage extract is at or below $10^{10}$ pfu/ml; wherein a crude bacteriophage extract having a total phage count of at least from about $10^{15}$ to about $10^{16}$ is obtained from about one liter of semi solid composition.

New claim 53 is supported in the specification; see [0034] of Patent Appl. Publ. No. 2007/0010001 A1.

New claims 54-57 recite that the extraction medium in step (c) of claim 1 comprises a sugar that reduces or abolishes bacterial phage-neutralizing activity, the sugar being present in the extraction medium at a concentration in the range of 0.2-2.0 M and being selected from the group consisting of N-acetyl-D-glucosamine, 2-deoxy-D-glucose, D-glucosamine, D-fructose, D-galactose, lactose, D-mannose, D-xylose, maltose, L-rhamnose, cellobiose, and sucrose, and more particularly from the group consisting of D-glucosamine, D-mannose, and L-rhamnose.

New claims 54-57 are fully supported in the specification; see [0123]-[0124] and Example 3 [0150]-[0158] of Patent Appl. Publ. No. 2007/0010001 A1.

New claims 58-59 recite that incubating the semi-solid culture medium is carried out at a temperature of about 37°C for a period of about 12 hours to about 24 hours, preferably about 14 hours to about 18 hours.

New claims 58-59 are supported in the specification; see [0098] of Patent Appl. Publ. No. 2007/0010001 A1.

Claim 11 was objected to under 37 CFR §1.75(c) as being of improper dependent form. In response, claim 10 is now canceled, and claims 11 and 12 are currently amended to depend from claim 1.

Claims 1-19, 44-49 and 51-52 were rejected under 35 USC §112, second paragraph, as being incomplete for omitting essential elements. In light of the current amendment of claim 1 to recite that a semi-solid culture medium is supported by a solid phase, withdrawal of this rejection is respectfully requested.

1-19, 44-49 and 51-52 were rejected under 35 USC §103(a) as being unpatentable over Swanstrom and Adams, *Proceedings of the Society for Experimental Biology and Medicine*, 1951 ("Swanstrom") in combination with U.S. Patent No. 6,322,783 to Takahashi ("Takahashi"); U.S. Patent Application Publication No. 2004/0091856 to Pelletier et al. ("Pelletier"); and U.S. Patent No. 6,482,632 to Agrawal ("Agrawal").

The present invention provides a method for commercial intermediate to large scale production of bacteriophage stock composition, in which each step is easy to perform and does not require large operating volumes. The method disclosed by Swanstrom discloses a semi-solid layer containing agar at a concentration of about 0.7% (page 372, column 2), well above the concentration of hydrocolloid concentrations of below 0.5% and 0.25-0.30% recited in [0020], [0026]-[0028] and [0092]-[0093] of Patent Appl. Publ. No. 2007/0010001 A1. This reduction in the hydrocolloid, e.g., agar, concentration in the top layer in accordance with the present invention not only significantly enhances the efficacy of bacteriophage extraction, but also produces a high bacteriophage titer of at least $10^{11}$, typically $10^{15}$ to $10^{16}$.

The Examiner asserted that even though Swanstrom uses a two-layer agar method of producing bacteriophage only on a small scale and since large scale quantities of bacteriophage are produced primarily through liquid fermenters, this reference is not considered to teach away from the present invention because the MPEP

states at section 2124 (II) that disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or nonpreferred embodiments.

Claim 1 has now been amended to include that (i) the hydrocolloid is at a concentration below 0.3% (ii) the crude bacteriophage extract is obtained by sequential serial extractions; (iii) the titer of the crude bacteriophage extract is at least $10^{11}$ pfu/ml; and (iv) the bacteriophage total yield is at least in the order of magnitude of $10^{15}$ to $10^{16}$ total pfu.

Support for hydrocolloid concentration of below 0.3% can be found in claim 4 (now canceled) and throughout the application. Support for the requirement of sequential serial extraction can be found in claim 9 (now canceled) and in [0034]-[0041] of Patent Appl. Publ. No. 2007/0010001 A1. Support for the bacteriophage titer and total yield can be found in claims 15 and 17, respectively, now canceled. Together, these steps of the method of the invention provide for the ability to produce bacteriophage compositions of high titer at a commercial scale, with high yield.

As stated in [0085] of Patent Appl. Publ. No. 2007/0010001 A1. "[t]he novel step in the method described in the present invention is the use of a semi-solid composition comprising hydrocolloid at a low concentration, specifically a concentration below 0.3%, preferably at a concentration of 0.25%-0.30%. This hydrocolloid concentration enables high replication rate of the phage, leading to a high titer of at least $5x10^{11}$ phage/ml, typically to a titer of $10^{12}$ phage/ml after multiple serial extractions..."(emphasis added). It should be noted that the applicant has found that the yield of *Pseudomonas aeruginosa* phage extracted from semi-solid medium comprising 0.6% agar was at least one order of magnitude lower compared to the yield obtained under the same conditions with a semi-solid medium comprising 0.27% agar. As exemplified in Patent Appl. Publ. No. 2007/0010001 A1 (Example 1, particularly paragraph [0141]), multiple serial extractions of the semi-solid medium provide equal volumes of highly concentrated (about $10^{10}$) crude bacteriophage extract without titer decrease for at least 11 subsequent extractions.

Nowhere in the cited background art is it disclosed or even suggested to upscale bacteriophage production by reducing the hydrocolloid concentration in the growth composition and by employing sequential serial extraction steps. Furthermore,

there is no suggestion that these steps would result in high yield and high titer of the resulted composition.

The method of Swanstrom, as well as slightly modified methods such as those described by Takahashi and Pelletier, have been found useful for preparations on a laboratory scale. For example, Swanstrom discloses (page 372, column 2) a 36 ml yield of crude phage extract, compared with 55 liters of crude phage extract in the present invention (Fig. 2A of Patent Appl. Publ. No. 2007/0010001 A1).

In the previous response, the applicant asserted that the combination of cited references teaches away from the claimed invention. In the recent Office Action, the Examiner disagreed, citing *In re Susi*, "Disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or nonpreferred embodiments."

The applicant respectfully reiterates his position that the cited prior art, Swanstrom in particular, teaches away from the method of the present invention. There is no suggestion whatsoever in Swanstrom that the described procedure is applicable to large scale production of bacteriophage stock compositions, and practical considerations preclude that possibility. Furthermore, as will now be discussed, Swanstrom's procedure differs from that of the present invention in several other significant respects.

Swanstrom discloses (page 374, column 1) that "Variation in the volume of base agar used per plate did not have an appreciable effect on phage yield over a range from 25 to 40 ml per plate, but a further reduction to 15 ml per plate did result in a decrease in yield....The layer of base agar serves as a source of nutrient for the dense population of bacteria growing in the soft agar layer."

Swanstrom employs agar in the amount of 2.5 ml per plate in the soft layer. Inclusion of agar in the amounts of 25 and 40 ml per plate in the base layer correspond to hard:soft layer ratios of, respectively, 10:1 and 16:1. Reduction in the amount of agar in the base layer to 15 ml per plate, which has a deleterious effect on phage yield, corresponds to a hard:soft layer ratio of 6:1.

By contrast, as disclosed in [0031] of Patent Appl. Publ. No. 2007/0010001 A1, the volume ratio of the supportive (hard) layer to the semi-solid (soft) layer in the

method of the present invention ranges from 2:1 to 10:1. Thus, Swanstrom teaches away from the low hard:soft layers ratios employed in the instant invention.

Swanstrom discloses (page 373, column 2 to page 374, column 1) that optimum phage yield was obtained by incubation for about 10 hours at a temperature of 37°C, and that the yield was reduced to ¼ of the maximum when the incubation period was extended to 24 hours.

By contrast, as disclosed in [0033] of Patent Appl. Publ. No. 2007/0010001 A1, incubation times at 37°C in the method of the present invention ranges from 12-24 hours, preferably 14-18 hours. Thus, Swanstrom also clearly teaches away from the incubation periods employed in the instant invention.

Agrawal is relied on solely for its teaching in Example 3 of a top layer containing a phage and 0.3% agar being formed on a base layer of a growth medium containing a variety of ingredients, including 1.2% agar. This medium appears to be designated as medium 3 at column 8, lines 39-42. The resulting plate is then incubated at 25-30°C for an undisclosed period of time. However it is recited at column 8, lines 28-30, that the incubation period employed with medium 3 is 3-6 days, a far longer period than that employed in the method of the present intention. Furthermore, there is no disclosure in Example 3 of Agrawal regarding the yield of phage so obtained.

From the foregoing remarks, it is clear that Agrawal fails to remedy the deficiencies in the teachings of Swanstrom and the other cited references as they relate to the present invention. The applicant respectfully asserts that the disclosures of Swanstrom, Takahashi, Pelletier, and Agrawal are not properly combinable to render obvious the method of the present invention. Thus, the method of the present invention would not have been obvious to one of ordinary skill in the art at the time the invention was made.

Withdrawal of the §103(a) rejection of the claims is therefore respectfully requested.

**Conclusion**

Claims 1-3, 5-8, 11-14, 16, 18-19, 44-49 and 51-59 are now in this case, whose prompt allowance is earnestly solicited Should there be any remaining questions or issues concerning this application, the Examiner is invited to contact the undersigned at the telephone number provided below.

The Commissioner is hereby authorized to charge any fees that may be due, or credit any overpayment of same, to Deposit Account No. 08-1935, Reference No. 2488.018.

Respectfully submitted,

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CERTIFICATE OF TRANSMISSION

I hereby certify that this correspondence is being transmitted by electronic filing to: Commissioner for Patents, Alexandria, VA 22313-1450, on March 2, 2009.

Jane M. Potts

Date of Signature: March 2, 2009